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THE BACTERICIDAL ACTION OF QUINONE AND OTHER PHENOL OXIDATION PRODUCTS AS DETERMINED BY THE RIDEAL-WALKER METHOD.*

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The method used in this work was the Rideal-Walker method for standardization of disinfectants.

The dilutions of the disinfectants to be tested were placed in ordinary wide mouthed test-tubes in five c.c. amounts and tested in series of 10 tubes each. One-half of a cubic centimeter of a 24 hour broth culture of *B. typhosus* was added to each of these test-tubes at 15 second intervals.[1] Six subinoculations of each of these were made into tubes containing 10 c.c. of broth, at intervals of 2½ minutes. The process of adding the culture to the mixing tubes containing the disinfectant occupied 2¼ minutes, allowing 15 seconds before it is necessary to make a subculture from tube number one. Proceeding then with subcultures at 15 second intervals, the time factor takes care of itself and we have a series of six subcultures from each mixing tube at intervals of 2½ minutes, i.e., from a 2½ minute exposure to the action of the disinfectant on the culture to one of 15 minutes. The tubes of broth were kept in racks to facilitate inoculation, and an assistant held up the tube to be inoculated. This assistant, however, is not necessary. The 15 second interval allowed was found to be ample, during which time the loop had to be flamed and allowed to cool. The inoculated tubes were then incubated at 37° C. for 48 hours, then read and tabulated. Plus (+) indicates growth, minus (−) no growth.

The material used.—All the glassware used was previously sterilized, as was also the distilled water for making the various dilutions of the disinfectants. The original dilutions were made by accurately weighing out the material on a chemical balance and

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[1] A definite measured amount of culture was added in preference to the drop method of Rideal and Walker.

diluting this in a standardized graduated flask, and the subsequent dilutions were made from this with standardized graduated pipettes. The loop used for subinoculations was two mm. in diameter and made of very fine platinum wire so that it would cool rapidly. A full loopful was transferred in every case.

The phenol coefficient was determined according to the Rideal-Walker method, namely: "by dividing the figure indicating the degree of dilution of the disinfectant that kills an organism in a given time by that expressing the degree of dilution of carbolic acid that kills the same organism in the same time under exactly similar conditions."[1]

There are two important, improved modifications of this method, as follows:

"The important modifications of the Lancet method on the Rideal-Walker are, in the increased number of dilutions employed, sometimes as many as 12 tubes being inoculated in each 2½ minute interval; extension of the number of time intervals to 30 instead of 15 minutes; the use of *B. coli* and MacConkey's bile salt media for subcultures instead of *B. typhosus* and standard extract broth; the amount of the mixture of culture and disinfectant transferred to the subculture tubes; the method of determining the coefficient.

"The coefficient, as determined by the Lancet Commission, is arrived at as follows: The figure representing the percentage strength of the weakest killing dilution of the phenol is divided by the figure representing the percentage strength of the weakest killing dilution of the unknown disinfectant, both at 2½ and at 30 minutes, the mean resulting figure being taken as the true coefficient. An example of the determination of the carbolic acid coefficient by the Lancet method may be seen from the following table:"

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[1] Taken from paper of Anderson and McClintic.

DISINFECTANT X.

Minutes	Dilutions							
	1-300	1-400	1-500	1-600	1-700	1-800	1-900	1-1,000
	Per cent 0.333	Per cent 0.250	Per cent 0.200	Per cent 0.166	Per cent 0.143	Per cent 0.125	Per cent 0.111	Per cent 0.100
2½	o	o	+	+	+			
5		o	o	+	+	+		
7½		o	o	+	+	+		
10			o	+	+	+	+	
12½			o	+	+	+	+	
15				+	+	+	+	+
20				o	+	+	+	+
25				o	+	+	+	+
30				o	+	+	+	+

CARBOLIC ACID CONTROL.

Minutes	Percentage Dilutions							
	1.10	1.00	0.917	0.846	0.786	0.733	0.687	0.647
2½	o	o	+	+				
5	o	o	+	+				
25					o	o	+	+
30					o	o	+	+

Room temperature 67° F.; + signifies growth; o signifies no growth; blank spaces signify not tested.

The coefficient is therefore:

$$\frac{\frac{1.00}{0.25}+\frac{0.733}{0.166}}{2}=\frac{4.0+4.4}{2}=4.2$$

The Hygienic Laboratory method uses a measured amount of 24-hour broth culture of *B. typhosus*, constant temperature in water bath for mixing tubes, a loop four mm. in diameter for making subinoculations, and the following modification of the Lancet method of determining the coefficient:

"After a large number of experiments we have concluded that the method employed by the Lancet Commission, with certain modifications, is the best one for determining the coefficient, i.e., the mean between the strength and time coefficients.

"In performing the test, plants are made every 2½ minutes up to and including 15 minutes. The method of determining the coefficient will be seen in table."[1]

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[1] Taken from paper of Anderson and McClintic.

Date, May 18, 1910.

Name "A."
Temperature of Medication, 20° C.
Culture Used, *B. typhosus*, 24 hr., Extract Broth, Filtered.
Proportion of Culture and Disinfectant, 0.1 cc.+5 c.c.
Organic Matter, None. Kind, None, Amount, None.
Subculture Media, Standard Extract Broth. Reaction, 1.5. Quantity in Each Tube, 10 c.c.

Sample	Dilution	Time Culture Exposed to Action of Disinfectants for Minutes:						Phenol Coefficient
		2½	5	7½	10	12½	15	
Phenol.............	1:80	−	−	−	+	+	+	
	1:90	+	−	−	−	+	+	80)375 4.69
	1:100	+	+	+	−	−	−	
	1:110	+	+	+	+	+	−	110)650
								5.01
Disinfectant "A"....	1:350	−	−	−	+	+	+	2)10.60
	1:375	−	−	−	+	+	+	5.30 =
	1:400	+	−	−	−	+	+	Coefficient
	1:400	+	−	−	−	+	+	
	1:425	+	+	−	−	−	−	
	1:450	+	+	−	−	−	−	
	1:500	+	+	−	−	−	−	
	1:550	+	+	+	−	−	−	
	1:600	+	+	+	+	−	−	
	1:650	+	+	+	+	+	−	
	1:700	+	+	+	+	+	+	
	1:750	+	+	+	+	+	+	

While making a series of tests for the standardization of some coal tar disinfectants, two samples of phenol were used; one had been in the laboratory a long time and had become slightly pink and the second was a recently purchased, colorless preparation. It was observed in a number of tests (see Table 1) that the colored phenol consistently showed a more powerful bactericidal action than the colorless phenol.

TABLE 1.

Date, February 12, 1911.

Sample	Dilution	Time Culture Exposed to Action of Disinfectants for Minutes:					
		2½	5	7½	10	12½	15
Phenol "N" (colorless).......	1-70	−	−	−	−	−	−
	1-80	−	−	−	−	−	−
	1-90	+	+	+	+	−	−
	1-100	+	+	+	+	+	+
Phenol "O" (light pink color)	1-70	−	−	−	−	−	−
	1-80	−	−	−	−	−	−
	1-90	+	−	−	−	−	−
	1-100	+	+	+	−	−	−
	1-110	+	+	+	+	+	−
	1-120	+	+	+	+	+	+

This suggested that, in this process of coloring, products of a stronger bactericidal action were formed, and that perhaps we would find this action increased with the increase in color in the phenol, and that these substances could be isolated and investigated separately.

H. D. Gibbs has shown that this process of coloring is an oxidation process and occurs in the presence of oxygen when phenol is exposed to sunlight and also slowly in the dark, but not when in contact with indifferent gases, such as hydrogen, nitrogen, and carbon dioxide. Also, that active oxygen will unite with phenol with considerable ease and rapidity, and ozone is very reactive. The principal substances formed are quinone, quinol, and catechol, and the principal colored compounds are probably quinone condensation products. The intense red product is probably phenolquinone.

We then secured three samples of colored phenol of different intensity and submitted them to the test. Sample "K" was a bright cherry red, phenol "N" was the colorless specimen, phenol "O" the slightly colored specimen originally tested, phenol "J" was intermediate between "O" and "K."

As Table 2 shows, there is an increase in the bactericidal action through phenols "O" and "J," but "K," which is the most colored, is but slightly stronger than "N," which is colorless.

TABLE 2.

Date, April 8, 1911.

Sample	Dilution	Time Culture Exposed to Action of Disinfectants for Minutes:					
		2½	5	7½	10	12½	15
Phenol "N" (colorless).......	1–70	−	−	−	−	−	−
	1–90	+	+	+	+	−	−
	1–100	+	+	+	+	+	−
	1–110	+	+	+	+	+	+
Phenol "O" (light pink color)	1–70	−	−	−	−	−	−
	1–90	+	−	−	−	−	−
	1–100	+	+	+	+	+	+
	1–120	+	+	+	+	+	+
Phenol "J" (color intermediate between "O" and "K")	1–90	−	−	−	−	−	−
	1–100	−	−	−	−	−	−
	1–110	+	+	−	−	−	−
	1–130	+	+	+	+	+	+
Phenol "K" (cherry red color)	1–70	−	−	−	−	−	−
	1–90	+	+	−	−	−	−
	1–100	+	+	+	+	+	+

As this may have been due to "K" being an inferior product and deficient in phenol, we next exposed to sunlight for different periods of time three samples of the colorless phenol "N," and obtained specimens of increasing degree of coloration. These were tested and the results shown in Table 3 were disappointing as they show no consistent increase of bactericidal action with increase in density of color of phenol or time of exposure. The only explanation we can offer is that the marked color present, due to these highly colored benzene compounds, must be caused by quantities insufficient to affect the test. There are, however, no chemical methods capable of testing this point.

TABLE 3.

Date, May 7, 1911.

Sample	Dilution	Time Culture Exposed to Action of Disinfectants for Minutes:					
		2½	5	7½	10	12½	15
Phenol "N" (desiccated)......	1-90	−	−	−	−	−	−
	1-100	+	+	−	−	−	−
	1-110	+	+	+	−	−	−
	1-120	+	+	+	+	+	−
	1-130	+	+	+	+	+	+
Phenol "N" (unexposed)	1-80	−	−	−	−	−	−
	1-90	+	−	−	−	−	−
	1-100	+	−	−	−	−	−
	1-110	+	+	+	+	−	−
	1-120	+	+	+	+	+	+
Phenol "N" (exposed April 14-18)....................	1-80	−	−	−	−	−	−
	1-90	+	+	−	−	−	−
	1-100	+	+	−	−	−	−
	1-110	+	+	+	+	+	−
	1-120	+	+	+	+	+	+
Phenol "N" (exposed April 14-26)....................	1-80	−	−	−	−	−	−
	1-90	+	−	−	−	−	−
	1-100	+	−	−	−	−	−
	1-110	+	+	−	−	−	−
	1-120	+	+	+	+	+	+
Phenol "N" (exposed April 14-May 7)...............	1-80	−	−	−	−	−	−
	1-90	+	−	−	−	−	−
	1-100	+	+	−	−	−	−
	1-110	+	+	+	+	−	−
	1-120	+	+	+	+	+	+

While waiting for the above sample to become colored we tested quinone and phenol-quinone, which were the only phenol oxidation products available to us, and also tested hydroquinone because of its close relationship to quinone as a reduction product. In view of the above these results were rather startling and are recorded in Table 4.

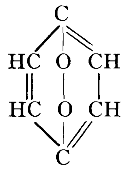
TABLE 4.

Date, April 11, 1911.

Sample	Dilution	Time Culture Exposed to Action of Disinfectants for Minutes: 2½	5	7½	10	12½	15	Phenol Coefficient
Quinone	1-600	−	−	−	−	−	−	100)16,000 160 = Coefficient (Rideal-Walker) 70)600 8.6 100)16,000 160.0 2)168.6 84.3 = Coefficient (Hygienic Lab.)
	1-1,000	+	−	−	−	−	−	
	1-1,500	+	−	−	−	−	−	
	1-2,000	+	−	−	−	−	−	
	1-2,500	+	+	−	−	−	−	
	1-3,000	+	+	+	−	−	−	
	1-4,000	+	+	+	−	−	−	
	1-4,500	+	+	+	−	−	−	
	1-5,000	+	+	+	−	−	−	
	1-10,000	+	+	+	−	−	−	
	1-16,000	+	+	+	+	+	−	
Hydroquinone	1-100	+	+	+	−	−	−	80)200 2.5 = Coefficient (Rideal-Walker)
	1-200	+	+	+	−	−	−	
	1-400	+	+	+	+	+	+	
Phenol-Quinone (freshly made and fresh solution)	1-800	−	−	−	−	−	−	70)800 11.4 100)6,000* 60.0 2)71.4 35.7 = Coefficient (Hygienic Lab.)
	1-1,000	+	−	−	−	−	−	
	1-1,500	+	+	−	−	−	−	
	1-2,000	+	+	−	−	−	−	
	1-4,000	+	+	+	+	−	−	
	1-8,000	+	+	+	+	+	+	
Phenol "N" (colorless)	1-70	−	−	−	−	−	−	90)4,000 44 = Coefficient (Rideal-Walker)
	1-90	+	+	+	+	−	−	
	1-100	+	+	+	+	+	−	
	1-110	+	+	+	+	+	+	

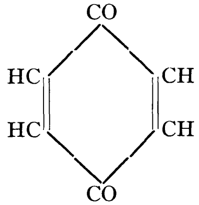
* Deduced from the table.

This table shows that the Rideal-Walker phenol coefficient of quinone is 160, which, as far as we have been able to discover, is the highest reported for any substance. Its formula is



Peroxide Formula
(1867, Graebe, *Z. f. Ch.*, N.F. 3, 39.)

or



Ketone Formula
(Fittig, A. 180, 23.)

and it is a very powerful oxidizing agent, its oxygen atoms being especially loosely bound, and its bactericidal action is undoubtedly to be attributed to this and not to its phenol moiety, for hydroquinone, with the two oxygen atoms replaced by hydroxyl groups, has a phenol coefficient of only 2.5.

It is also interesting to note that the activity of quinone is reduced when it is joined with the less active phenol to form phenol-quinone (coefficient 44). This latter is an unstable compound in solution and breaks down readily on standing, forming a muddy fluid. Quinone, which has a penetrating, irritating odor, is more stable in solution.

Our experience with the Rideal-Walker method has demonstrated to us that it has the defects pointed out by Anderson and McClintic of the Hygienic Laboratory and by the Lancet Commission. The most serious of these, as is shown by the above tables, is the variation of the bactericidal action directly with the increase in temperature. These tests were made at room temperature and very definitely demonstrate an increased bactericidal action with the approach of warm weather. The comparison of the activity of the disinfectant has consequently always been made with that of the standard phenol similarly tested at the same time under exactly similar conditions. Therefore the phenol coefficients determined at different times are comparable one with the other. In the choice of the dilutions for calculating the phenol coefficient, too much variation is allowed by the choice and personal equation of the investigator.

While the tables show some irregularity, we do not believe it sufficient to vitiate the results, especially as we have been able to closely confirm them by a number of repetitions of the work. Whereas one probably cannot exactly fix a phenol coefficient with the above method one can certainly determine the comparative germicidal efficiency of a series of disinfectants.

From the above it seems clear that very little, if any, increase in the bactericidal action occurred in the phenol exposed to the sun and also that quinone and phenol-quinone are many times more active than phenol; quinone being the most active substance known.

Our work was begun with the Rideal-Walker method and completed with the same for the sake of consistency of results. The Hygienic Laboratory phenol coefficient method we believe is a distinct advance over that of the Lancet Commission and we intend using it in any future work.

In the articles by Anderson and McClintic and the Lancet Commission there is a complete review of the progress of study of the standardization of disinfectants.

In conclusion we wish to take this opportunity of expressing our gratitude to Dr. J. H. Kastle for his kind interest and our indebtedness for his many suggestions which have guided us in this work. We wish also to express our indebtedness to Mr. Etheridge, of the Second Year Medical Class, for his assistance in the tests.

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